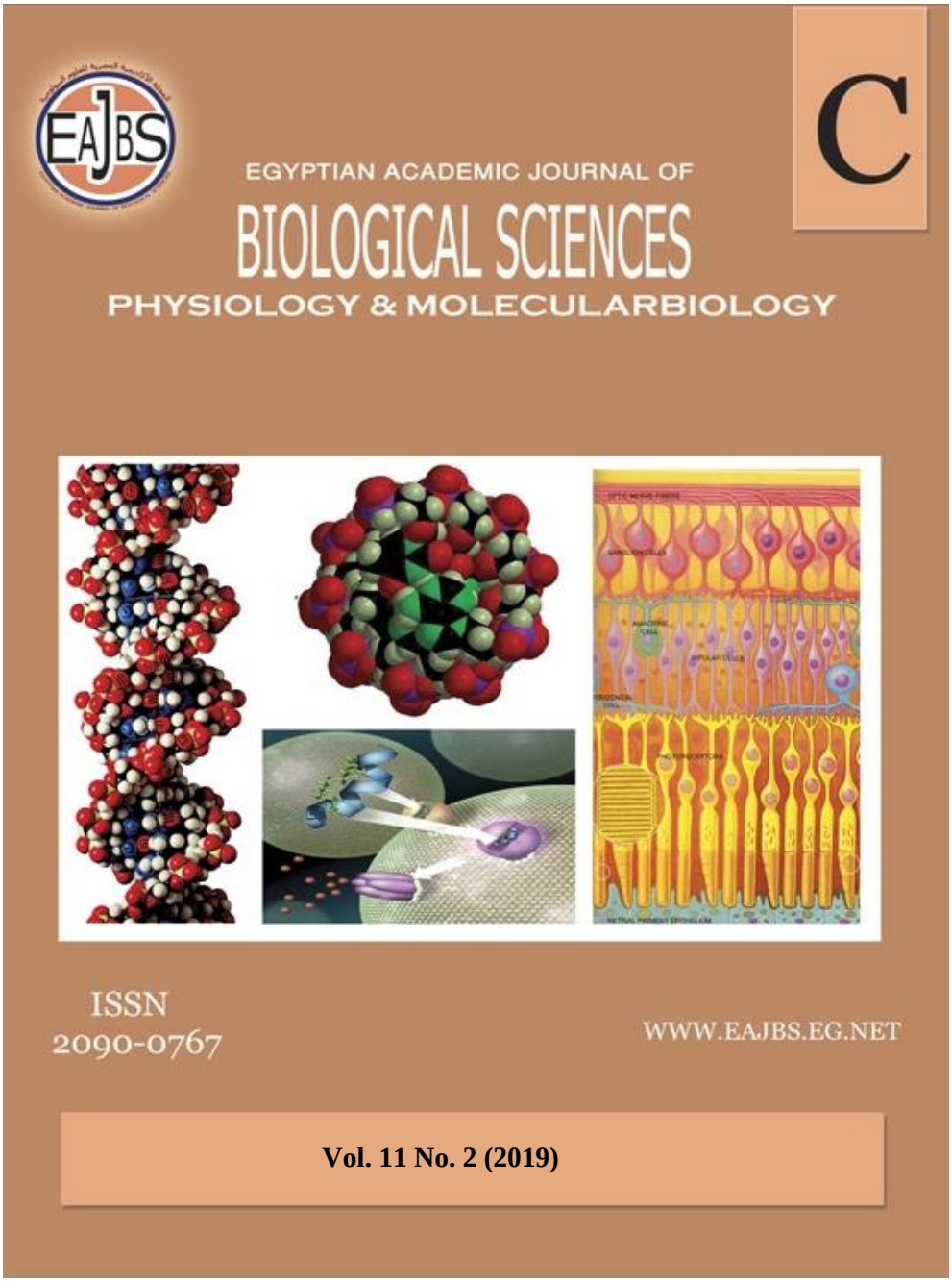

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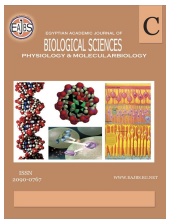

Effect of Selenium and Bee Pollen against Immunotoxicity and Hepatotoxicity Induced by Cadmium in Male Albino Rats.

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ABSTRACT

Cadmium is a toxic naturally occurring element that affects numerous organ systems in humans, the present study was designed to study the role of bee pollen and selenium against cadmium chloride induced immunotoxicity and Hepatotoxicity in male albino rats.

Forty (40) male albino rats were used for the experiment. Group I was intraperitoneal (i.p.) Injected with saline solution, G2 (i.p.) Injected with CdCl2 (1 mg/kg b.w.), G3 were (i.p.) Injected with CdCl2 (1 mg/kg b.w.) and then receive selenium (i.p.) Injection at a dose of (0.5 mg/kg/day). G4 (i.p.) Injected with CdCl2 (1 mg/kg b.w. day by day) and then received bee pollen orally at a dose of (10 g/kg/day). Animals were exposed to treatment once daily for 10weeks. After the last day of treatment, animals were sacrificed and blood samples collected for hematological analysis and serum separated for serum liver enzymes analysis and the liver separated for oxidative stress analysis. Results obtained in this present study revealed a significant increased at ($p>0.05$) in WBCs, Monocyte , Lymphocyte , Granulocyte and The activities of IgG and IgM, aspartate aminotransferase (AST), alanine transferase (ALT) ,alkaline phosphatase (ALP), (Total- Direct) bilirubin and Malondialdehyde (MAD), while showed a significant decreased at ($p>0.05$) in albumin and protein, Glutathione (GSH), Superoxide dismutase (SOD) and Catalase (CAT) in group treated with CdCl2 only when compared with normal group. However, Effects of bee pollen and selenium on immunotoxicity and hepatic injury due to cadmium-induced oxidative stress revealed a significant decreased at ($p>0.05$) in WBCs, Monocyte, Lymphocyte and The activities of IgG except Granulocyte and IgM revealed an insignificant decreased also aspartate aminotransferase(AST), alanine transferase (ALT) ,alkaline phosphatase (ALP), (Total- Direct) bilirubin and Malondialdehyde (MAD) showed a significant decreased at ($p>0.05$), while Total protein, Albumin, Glutathione (GSH), Superoxide dismutase (SOD) and Catalase (CAT) showed a significant increased at ($p>0.05$) when compared with control group. It is concluded that the bee pollen and selenium have antioxidant properties.

INTRODUCTION

Toxicity due to overwhelming metals like lead, cadmium, mercury and so on is all around recorded in the literature. (Leonard *et al.,* 2004). Mechanism of heavy metal toxicity may be multifactorial. One of the important mechanisms behind toxicity of heavy metal has been ascribed to the generation of the reactive oxygen species (ROS) by heavy metal which leads to oxidative stress induced organ injury. Studies presented an indication that metals may interact with nuclear proteins and DNA causing oxidative stress of biological macromolecules (Flora *et al.,* 2004). Friberg *et al.,* (1992) reported that cadmium (Cd) is heavy metal, it known to be present in water, air, food and even cigarette smoke. Also, it is an industrial pollutant from battery, plastic and fertilizer industries (Stohs and Bagchi, 1995). Reactive oxygen species (ROS) are an inescapable result of cell breath causing oxidation of lipids, nucleic acids and proteins, and ROS harm is a basic reason for illness, including malignancy, inflammatory, and neurodegenerative sicknesses (Cadet *et al.,* 2005; Valko *et al.,* 2006 and de Flora *et al.,* 2007). Several researches have indicated that Cd can stimulate the formation of reactive oxygen species (ROS) and induce damage to various tissues (Alvarez *et al.,* 2007and Thompson and Bannigan, 2008) it is One of the most dangerous toxins for the environment and occupational toxins. It is found in drinking water in food and atmospheric air due to its use in industry (Buchet *et al,* 1990). Cadmium is accounted for to be extremely dangerous to biological system, the erythrocytes, kidney, brain, liver and testes are considered, to the most susceptible in the case of exposure to cadmium become this heavy metal aggregate in these cells of these organs (Jarup *et al.,* 1998). Heavy metals are generally immunotoxin, among the many effects displayed (Villanueva *et al.,* 2000) Also cadmium particularly, has been shown to initiate issue in the humoral and cellular immune responses. (Kataranovski *et al.,* 1998; Dan *et al.,* 2000 and Marth *et al.,* 2001).

Protection of cells from Cd-induced oxidative stress caused by ROS and free radicals is in the form of both enzymatic and non-enzymatic defense mechanisms existing in the cell (Droge, 2002), in current years several compounds with antioxidant properties have been found to be beneficial and may protect or restore physiological function in heavy metals-induced oxidative damage. So Antioxidants ameliorate oxidative harm caused by ROS, and research has concentrated on the role of antioxidants for the treatment and prevention of disease.

Accordingly, natural products and customary medicines with developed efficacy and safety profiles are needed as a substitute for chemical therapeutics. It is represented that several of natural products have been appeared to protect against liver damage, and many possess one or a combination of antioxidant, immune modulatory, antifibrotic or antiviral activities (Seeff *et al.,* 2001; Lee and Jeong, 2002 and Shin *et al.,* 2006). Selenium (Se) utilized as nourishing supplements, is the fundamental components in practically all biological systems. Yuan and Tang, (1999) and Akhtar *et al.,* (2009) detailed that Se has the ability to counteract free radicals and ensure the structure and capacity of proteins, DNA and chromosomes against oxidation damage This protection includes the capability of Se to alter the distribution of Cd in tissues and induces binding of the Cd-Se complexes to proteins, which are similar to metallothioneins

(Jamba *et al.,* 1997and Combs and Gray, 1998). Also, Bee products are thought of to be a possible supply of natural antioxidants capable of counteracting the consequences of oxidative stress causative the pathologic process of diverse diseases. It is believed that the bee products are massive sources of antioxidants also bee pollen is one in every of the richest and purest natural foods that way ever discovered;

the tremendous nutritional and healthful worth of the pollen has been used for hundreds of years. Bee pollen is a perfectly well-adjusted food and its contents is rich in enzymes, amino acids, hormones, proteins, minerals, fats, carbohydrates, a considerable amount of, phytochemicals, vitamins, phenolic substances and significant quantities of antioxidant agents (Bogdanov, 2004; Eraslan, *et al.*, 2010 and Hegazi, 2012). The chemical composition of the bee pollen depends on its botanic and geographical properties. Pollen contains regarding 1–5% total phenolic substances that embody totally different subtypes like flavonoids, anthocyanins, tannins and phenolic acids. They exhibit a good range of biological activities as well as antioxidant, anti-inflammatory, antiatherogenic, antimicrobial, antithrombotic and anticarcinogenic activities (Nagai *et al.*,2001; Eraslan, 2009; Eraslan, *et al.*, 2010 and Hegazi, 2012) So The aim of this study was to investigate the oxidant status in cadmium chloride (CdCl2)-induced hepatotoxicity change and the antioxidant effect of selenium and bee pollen supplementation on the experimental model (Rats).

MATERIALS AND METHODS

Materials

1-Animals

40 adult male albino rats weighting approximately **(200±20g)** , They were allocated in stainless steel cages in an automatically, illuminated and thermally controlled room (22- 25°C and 12 hrs light / dark cycle) at the Animal House, Faculty of Science, south valley University, Qena, Egypt. They fed on adequate stable commercial balanced diet.

2-Experimental Treatments:

After the acclimation period of one week, the experimental animals were divided at random into 4 groups 10 animals of each group.

G1 (Normal group): rats were intraperitoneal (i.p.) Injected with only saline solution (NaCl 0.9%), this group served as a normal group for 10 weeks.

G2 (Control group): rats were intraperitoneal (i.p.) Injected with $CdCl_2$ (1 mg/kg b.w.) For 10 weeks.

G3 the third group: rats were intraperitoneal (i.p.) Injected with $CdCl_2$ (1 mg/kg b.w.) and then received selenium (Na_2SeO_3) intraperitoneally (i.p.) Injection at a dose of (0.5 mg/kg/day) for 10weeks.

G4 the Fourth group: rats were intraperitoneal (i.p.) Injected with $CdCl_2$ (1 mg/kg b.w. day by day) and then received bee pollen orally at a dose of 10 g/kg/day for 10weeks.

At the end of the experimental period, rats were sacrificed under ether anesthesia. Blood for hematological analysis was collected in tubes containing EDTA as an anticoagulant, another Blood samples were collected in clean dry non-heparinized centrifuge tubes. Sera were separated by centrifugation at 4000 rpm for 20 min, and frozen at -20°C for future biochemical analysis.

Liver Tissue Was Homogenized as Following:

a) Prior to dissection, perfuse tissue with phosphate buffer saline (PBS) solution pH=7.4 containing 0.16 mg/ml heparin to remove any red blood cells and clots.

b) Homogenize the tissue in 5-10 ml cold buffer (i.e., 50 mM potassium phosphate, pH7.5 1mM EDTA and 1gm⁄100ml of poly phenylpyridine) per gram tissue in pastel homogenizer.

C) Centrifuge at 4000 rpm for 15min at 4^oc.

d) Remove the supernatant for assay and store on ice. If not assaying on the same day, freeze the supernatant at - 80^oc, the sample will be stable for at least one month.

Methods

The hematological evaluation consisted of white blood cells (WBCs), Monocyte, Lymphocyte and Granulocyte using the automatic machine (coulter counter). Determination of IgG and IgM were determined by (ELIZA) method. All

biochemical parameters (GPT, GOT, ALP , (Total- Direct)Bilirubin, protein and albumin, MDA, and antioxidant enzymes (GSH and CAT) were determined using available kits brought from Bio-diagnostic Co. Giza .Egypt.

Determination of Serum (GPT and GOT) was determined by a colorimetric kinetic method described by (Reitman and Frankel, 1957). The level of Serum Alkaline Phosphates (ALP) was determined by the colorimetric method using EC 3.1.3.1 kits which obtained from, Bio-diagnostic Co. Giza, Egypt. This method described by (Bolfield and Goldberg, 1971). And protein level was detected according to the Biuret method described by (King and Wooton., 1964). While albumin concentration was determined in serum according to the method of (Doumas *et al.*,1971). According to the method of (Walter and Gerade, 1970), direct bilirubin concentration was also determined in serum, while total bilirubin was determined according to (Schmidt and Eisenburg,1975). Determination of MDA was carried out according to the method of (Ohkawa et al., 1979), while GSH was determined by the colorimetric method described by (Beutler *et al.*, 1963), and CAT was determined by the colorimetric method described by (Aebi, 1984).

Statistical Analysis:

The obtained data were expressed as mean ± standard deviation (SD). All data were analyzed statistically using one-way analysis of variance (ANOVA) The results were considered statistically significant when $p < 0.05$.Statistical Package for Social Sciences (SPSS) for Windows version 12.0 software was used for this analysis.

RESULTS

A - Immune Analysis.

1- The Effect of Cadmium Chloride (CdCl2) (l mg/kg B.W.), Selenium(0.5mg/kg B. W.) and pollen (10g/kg B. W.) on WBCs, Monocyte, Lymphocyte, Granulocyte and The Activities of IgG and IgM in Male Albino Rats Post-Injected With Cadmium Chloride (CdCl2) (l mg/kg B.W.).

The results are recorded in table1 showed a significant increase in WBCs, Monocyte, Lymphocyte, Granulocyte and the activities of IgG and IgM at ($p<0.05$) in rats injected with cadmium chloride (CdCl2) when compared with normal group. an improvement effect observed in the previous parameters when the experimental rats were given Selenium(0.5mg/kg B. W.) after injected with cadmium chloride (CdCl2) which indicated by a significant decrease when compared with control animals and it became near to normal animal except Granulocyte, IgG and IgM not reach to normal animals. Significant changes were observed as indicated in table 1 the WBCs, Monocyte, Lymphocyte and the activities of Igg showed significant decrease at ($p<0.05$) while Granulocyte and IgM showed an insignificant decrease in rats injected with cadmium chloride (CdCl2) given pollen (10g/kg B. W.) when compared with the control animals.

B- Biochemical Analysis:

Liver Functions:

2- The Effect of Cadmium Chloride (CdCl2) (l mg/kg B.W.), Selenium (0.5mg/kg B. W.) and Pollen (10g/kg B. W.) on Serum GPT, GOT, ALP, (Total- Direct) Bilirubin Activities, Protein and Albumin Level in Male Albino Rats Post Injected With Cadmium Chloride (CdCl2) (l mg/kg B.W.).

As recorded in table (2) hepatic injury induced by cadmium chloride ($CdCl_2$) caused a significant increase in the enzyme's activities (GPT, GOT, ALP and (Total and Direct) Bilirubin) while albumin and protein showed a significant decrease when compared with the normal rats. Significant changes were observed when the experimental rats injected with cadmium chloride given Selenium (0.5mg/kg B. W.) as indicated in table 2 the serum activities of GPT, GOT, ALP and (Total and Direct Bilirubin) showed a significant decrease while Albumin and protein showed a significant increase when compared with the control animals.

Also when the experimental rats injected with cadmium chloride given pollen (10g/kg B. W.) showed the ameliorative effect on the hepatic injury induced by cadmium chloride which indicated by the significant decreases in activity of GPT, GOT, ALP and (Total-Direct)Bilirubin), and a significant increase in the albumin and protein activities were when compared with the control animals . But when the treated animals compared with the normal rats the results are recorded revealed that GPT, GOT, ALP and (Direct Bilirubin) activities were still significant increases but the albumin, protein and (Total Bilirubin) activities improved and become near to normal**.**

C- Liver Homogenate Biochemical Analysis.

3-The Effect of Cadmium Chloride (CdCl2) (l mg/kg B.W.), Selenium(0.5mg/kg B. W.) and Pollen (10g/kg B. W.) on Lipid Peroxidation Malondialdehyde (MDA), Glutathione (GSH) and Catalase Concentration of Liver Tissue in Male Albino Rats Post Injection With Cadmium Chloride (CdCl2) (l mg/kg B.W.).

The results recorded in Table 3 indicated that the concentration of liver MDA was significant increased associated with significantly decreased in GSH and catalase activities in rats injected with cadmium chloride (CdCl2) when compared with normal rats. While the administration of Selenium(0.5mg/kg B. W.) and Pollen (10g/kg B. W.) with CdCl2 caused a significant decrease of liver MDA, on the other hand GSH and catalase were significant increase when compared with control animals. But when compared with the normal rats the results indicated that MDA was still significant increases but GSH and catalase concentration were improved and become near to normal animal.

Table(1): The effect of cadmium chloride (CdCl2) (l mg/kg B.W.), Selenium(0.5mg/kg B. W.) and pollen (10g/kg B. W.) on WBCs, Monocyte, Lymphocyte, Granulocyte and The activities of IgG and IgM in male albino rats post-injected with cadmium chloride (CdCl2) (l mg/kg B.W.).

Groups / Parameters	Normal group Mean ± S.D.	Control group rats injected with (cadmium chloride $CdCl_2$) Mean ± S.D.	Group (3) Rats injected with (cadmium chloride + selenium) Mean ± S.D.	Group (4) Rats injected with (cadmium chloride + bee pollen) Mean ± S.D.
WBCs(10^3/mm^3)	8.11 ± 0.58	11.28 ± 1.26^{+a}	9.85± 1.02 $^{+a-b}$	10 ± 0.89 $^{+a-b}$
GR (10^3/mm^3)	5.16 ± 0.58	6.43 ± 0.97^{+a}	6.11 ± 1.20 $^{+a}$	6.31± 1.04 $^{+a}$
MO(10^3/mm^3)	1.32 ± 0.17	1.92 ± 0.26^{+a}	1.65 ± 0.17 $^{+a-b}$	1.72 ± 0.22 $^{+a-b}$
LY (10^3/mm^3)	1.71 ± 0.17	2.95 ± 0.33 $^{+a}$	2.11 ± 0.38^{+a-b}	2.04 ±0.38^{+a-b}
IgG(g/l)	196.20 ± 19.70	349± 23.94 $^{+a}$	246.6 ± 8.04^{+a-b}	256.6 ± 53.28^{+a-b}
IgM(g/l)	17.7 ± 4.15	31.20 ± 5.43^{+a}	29.5 ± 7.07^{+a}	19.31 ± 4.50 $^{-b}$

The results presented the mean ± S.D. of 10 rats

+significant increase at ($p<0.05$). a → significantly different from normal rats.

–significant decrease at ($p<0.05$). b → significantly different from control rats.

Table(2): The effect of cadmium chloride (CdCl2) (l mg/kg B.W.), Selenium(0.5mg/kg B. W.) and pollen (10g/kg B. W.) on serum GPT, GOT, ALP , (Total-Direct)Bilirubin activities, protein and albumin level in male albino rats post-injected with cadmium chloride (CdCl2) (l mg/kg B.W.).

Groups / Parameters	Normal group Mean ± S.D.	Control group rats injected with (cadmium chloride $CdCl_2$) Mean ± S.D.	Group (3) Rats injected with (cadmium chloride + selenium) Mean ± S.D.	Group (4) Rats injected with (cadmium chloride + bee pollen) Mean ± S.D.
GPT (U/ml)	32.6 ± 3.09	59.9 ± 9.3 $^{+a}$	47.15± 5.8 $^{+a-b}$	43.8 ± 7.03 $^{+a-b}$
GOT (U/ml)	142.6 ± 13.9	239.8 ± 40.6 $^{+a}$	157.6± 15.04 $^{-b}$	167.9 ± 12. 4 $^{+a-b}$
ALP(U/ml)	133.5 ± 24.4	186.2 ± 35.12 $^{+a}$	126.5 ± 23.8 $^{-b}$	158.2 ± 33.5 $^{+a-b}$
Total_Bilirubin	0.296 ± 0.108	5.78 ± 1.91 $^{+a}$	0.361± 0.118 $^{-b}$	0.438 ± 0.366 $^{-b}$
Direct_Bilirubin	0.030 ± 0.010	0.835 ± 0.394 $^{+a}$	0.296 ± 0.060 $^{+a-b}$	0.309 ± 0.092 $^{+a-b}$
Protein (g/ dl)	7.19 ± 0.35	6.3 ± 0.40 $^{-a}$	7.27 ± 0.42 $^{+b}$	7.46 ± 0.53 $^{+b}$
Albumin (g/dl)	2.79 ± 0.32	2.07 ± 0.41 $^{-a}$	3.34± 0.27 $^{+b}$	3.005 ± 0.36 $^{+b}$

The results presented the mean ± S.D. of 10 rats

\+ significant increase at ($p<0.05$). a → significantly different from normal rats.

– significant decrease at ($p<0.05$). b → significantly different from control rats.

Table 3: The effect of cadmium chloride (CdCl2) (l mg/kg B.W.), Selenium(0.5mg/kg B. W.) and Pollen (10g/kg B. W.) on lipid peroxidation malondialdehyde (MDA), Glutathione (GSH) and Catalase concentration of liver tissue in male albino rats post injection with cadmium chloride (CdCl2) (l mg/kg B.W.).

Groups / ***Parameters***	Group (1) Normal rats Mean ± S.D.	Group (2) Control Group Rats injected with (cadmium chloride) Mean ± S.D.	Group (3) Rats injected with **(cadmium chloride** + selenium) Mean ± S.D.	Group (4) Rats injected with **(cadmium chloride** + pollen) Mean ± S.D.
MDA (nmol/ g.tissue)	8.05 ± 1.76	12.72± 2.10 $^{+a}$	9.73 ± 1.34 $^{+a-b}$	10.5 ± 1.26 $^{+a-b}$
GSH (mmol/ g.tissue)	2.04 ± 0.245	0.74 ± 0.09 $^{-a}$	2.11± 0.52 $^{+b}$	2.12 ± 0.245 $^{+b}$
Catalase (mmol/ g.tissue)	0.90 ± 0.096	0.63 ± 0.24 $^{-a}$	0.92 0.045 $^{+b}$	0.94 ± 0.009 $^{+b}$

The result presented the mean ± S.D. of 8 rats

\+ Significant increase at ($p<0.05$). a → significantly different from normal rats.

– Highly significant decrease at ($p<0.05$) b → significantly different from control rats.

DISCUSSION

Recently a lot of attention has been focused on protective role and mechanism of action of naturally occurring compounds in the biological system. Selenium and Bee pollen and other bioactive compounds is known to have

antioxidative effects (Toufektsian *et al.,* 2000; Leja *et al.,* 2007 and Ramoutar *et al.,* 2007).

In the present work, we have to study the effect of Bee pollen and Selenium on liver damage and immunotoxicity induced by cadmium chloride (CdCl2).

Cadmium is one in all the foremost harmful industrial and environmental serious metals, to which humans are exposed through contaminated foods, water. Chronic cadmium poisoning can result in nephrotoxicity, cardiovascular diseases, osteoporosis and testicular necrosis, prostatic and testicular cancers (Oteiza *et al.,* 1999 and Shaikh and Tang, 1999). Its intracellular accumulation makes oxidative stress resulting in hepatocellular injury via displacement of redox-active metals, depletion of redox scavengers, inhibition of anti-oxidant enzymes and inhibition of the electron transport chain leading to mitochondrial destruction (Patra *et al.,* 2011; Adiele *et al.,* 2012 and Nair *et al.,* 2013).

Heavy metals are an outstanding category of immunotoxins connected with each specific and nonspecific immunoenhancement and immunosuppression. As a result of this capacity to change immune system homeostasis, some heavy metals have been implicated as causative agents or aggravating factors in the improvement of compound hypersensitivity, allergy, and autoimmune disease or in increased susceptibility to infections (Druet, 1995; Lawrence and McCabe, 2002 and Peden, 2002). Be that as it may, the immunotoxin properties of most heavy metals are not all around reported, and in all cases, the underlying mechanisms are only poorly understood.

Pillet *et al*., (2006) reported that the mice treated with Cd chloride which caused depth changes in immune system. Likewise, Cd immunotoxicity has been reported in humoral response after chronic exposure (Blakley *et al.,* 1985) the immune-sensitizing and immune-stimulating properties of cadmium and lead are still not understood. In fact, much of the existing data actually suggest a suppressive role for both these metals on immune function (Cook *et al.,* 1975; Fujimaki *et al.,* 1983; Descotes, 1992; Fischbein *et* al., 1993; Ritz *et al.,* 1998 and Sarasua *et al.,* 2000).

In the present study rats were intraperitoneal (i.p.) Injected with CdCl2 (1 mg/kg b.w.) for 10 weeks showed a significant increase in WBCs, Monocyte, Lymphocyte and Granulocyte at ($p<0.05$) when compared with normal group. Those findings were agreed with the results of previous studies (Horiguchi, 2007 and Shata *et al.,* 2014). Christensen *et al.,* (1978) reported that in fish, total leucocytes reacted to totally different stressors together with infections and chemical irritants. The significant increase of lymphocytes and the white blood cells were detected in fish exposed to CdCl2. Increased WBCs has been recommended because of stimulated lymphopoiesis and enhanced release of lymphocyte from lymphoid tissues. Such lymphocyte response in the presence of poisonous substances may be related to pollutant-induced tissue injury and severe disturbance of the non-specific immune system leading to increased production of leucocytes (Das and Mukherjee, 2003). Also, Yagminas *et al.,* (1990) showed that the leukocytosis has been attributed to the lead-induced inflammation. The relationship between antioxidant and immune cells may be explained with the fact that the immune cell function is linked to the release of Reactive Oxygen Species (ROS) even though this ROS is involved in the microbial and cytotoxic activity of immune

cells, excessive amount of it is harmful for immune cells as it attack cellular components and lead to cell damage or death. Immunoglobulins are synthesized by plasma cells and to some extent by lymphocytes. Five classes of immunoglobulins have been recognized – IgG, IgA, IgM, IgD, and IgE from them IgG, IgA and IgM are major and IgD and IgE are minor immunoglobulins.(Ananthnarayana and Paniker, 2005) In the present study, we demonstrated that the immunoglobulin profile of IgM and IgG was significantly increased in CdCl2-treated rats. These results agree with Wen *et al.,* (2015) showed that Hyperimmunoglobulinemia is often discovered in patients with chronic liver diseases. However, the precise mechanism underlying the high level of antibody formation is not fully understood, they deliver indication for the functional role of the liver and also the stimulation of plasma cell production in hyperimmunoglobulinemia. The liver is an organ with major innate immunity and acts as an organ wall or a filter between the gastrointestinal tract and the remainder of the body. Intrinsic immunity is additionally concerned within the pathogenesis of liver cirrhosis (Racanelli and Rehermann, 2006). The exact mechanism underlying the high levels of antibody-formation are isn't absolutely understood. Previous studies show that hyperimmunoglobulinemia in the systemic circulation is most closely related with chronic HBV infection (Tsai *et al.,*1990; Tsai *et al.,* 1998 and Abbott *et al.,*2007). Wen *et al.,* (2015) showed that the chronic liver diseases also showed high levels of serum immunoglobulin, which means that the occurrence of high levels of immunoglobulin is related with factors or mechanisms other than HBV infection. Triger and Wright, (1973) pointed to the damaged liver could lose its filtering capability and permit several gut antigens and endotoxins to access the immune system, which might lead to the augmented synthesis of immunoglobulins.

The ROS can be scavenged by antioxidants leading to improve immune cell functions (De la Fuente and Victor, 2000; Victor *et al*., 2003 and Barillari *et al.,* 2005). Antioxidants are very essential to immune defense and animal health, consequently, to their production capacity (Chew, 1995). Natural antioxidant was found to stimulate cell-mediated immunity of rabbits (El-Kholy *et al*., 2008).In the present study the administration of selenium(0.5mg/kg B. W.) and pollen (10g/kg B. W.) after injected with cadmium chloride (CdCl2) which indicated a significant decrease in WBCs, Monocyte, Granulocyte and The activities of IgG when compared with control animals, This result confirmed by the findings of Mgbenka *et al.,* (2004) who founded that the increase in mean weekly of total WBC, of rats given diet with selenium than the non-supplemented rats indicated that selenium had an impact on the immune system of the rats. The increasing of WBCs during a bound level is a good indicator of increasing immunity efficiency. This result confirmed by the findings of (Attia *et al.,* 2014) for broiler chicks and (Hedia *et al.,* 2007; Attia *et al.,* 2014 and El-Neney and El-Kholy , 2014) for rabbits. WBCs were increased significantly this is a good indicator of the increase in immune efficiency (Swiderek *et al*., 2006). Bee-pollen can enhance immunity (Song *et al.,* 2005; Liu *et al.,* 2010 and Attia *et al.,* 2011). Bee pollen can improve the cellular immune responses, antibody production speed and reinforce the immunological system (Song *et al.,* 2005). Enhancements

within the immune response might be due to including of antioxidant and minerals, which present in vitamins and flavonoids in bee-pollen, which have a role in enhancing the immune system.

In the present study, we founded that the injection of $CdCl_2$ (1 mg/kg b.w.) showed that the activities of serum GPT, GOT and ALP were significantly increased when compared to normal levels. Liver enzymes in serum e.g., GPT, GOT and ALP are mainly used in the evaluation of hepatic damage. So The increasing of serum GPT, GOT and ALP could be resulting in elevated release and escape out of these enzymes from the liver cytosol into the blood stream may be resulted in alteration in the permeability of the liver membrane takes place, also The elevated activities of these enzymes due to ALP is a membrane-bound enzyme and is released unequally depending on the pathological phenomenon which provides a sign of the liver damage effect of this metal (Pari and Murugavel, 2005). Transaminases (GPT and GOT) play a very important role in amino acids catabolism and bio-synthesis. They are responsible for detoxification processes, metabolism and biosynthesis of energetic macromolecules for various important functions (Aly *et al.,* 1997; Awad *et al.,* 1998 and Seven *et al.*, 2004). Similarly, GPT and GOT were elevated in rats intraperitoneally injected with cadmium at a dose of 2mg/kg/day for 8 days (Meral *et al*., 2007).

Albumin has many important functions, such as maintaining plasma colloid osmotic pressure, anti-oxidation and substances transfer (Schoentgen *et al.,* 1986; Haefliger *et al.,* 1989 and Sugioet *et al.,* 1999). Proteins are necessary organic substances needed in tissue binding and repair, under extreme stress conditions protein supplies energy in metabolic pathways and biochemical reaction reported the (Senthilkumar *et al.,* 2007).In the present study, we founded that the injection of cadmium chloride ($CdCl_2$) (1 mg/kg b.w.) showed a significant decreases in total protein and serum albumin, compared to their normal levels. Normally, the reduction of serum proteins and albumin levels as shown in Table 1 indicates liver disease. This reduction could be attributed to the changes in the metabolism and the synthesis of the protein and free amino acid in the liver (Li *et al.,* 2007)The significant decrease in serum total protein and albumin may also be recognized as impairment of hepatocyte functions causing reduced cytochrome activity P-450 and inhibition of protein metabolism in the liver. (Ibiam *et al.,* 2013; Asagba, 2010).

In this study, we founded that the injection of cadmium chloride ($CdCl_2$) (1 mg/kg b.w.) showed a significant increase in Total Bilirubin and Direct Bilirubin when compared to their normal levels. Raise in bilirubin level may be either because of elevated RBCs and/or haemoglobin breakdown proved to type the haematological alterations, the rise in plasma total bilirubin may result from decreased liver uptake, conjugation or increased Bilirubin production from hemolysis (Rana *et al*., 1997). or because of hepatic injury showed from the irregular serum biochemistry. It was indecated that increase in serum bilirubin is related with free radical releasing induced hepatic damage (Sedlak and Snyder, 2004).

Malondialdehyde (MDA) is a terminal product of the lipid peroxidation process and the determination of levels of MDA provides a good measure of lipid peroxidation, one of the main mechanisms of cell injury leading to

necrosis or apoptosis. (Chlubek *et al.*, 2003).In the present study we indicated that the injection of cadmium chloride ($CdCl_2$) (1 mg/kg b.w.) showed a significant increase of the level of lipid peroxidation product, MDA, within the liver tissue of rats, that were accompanied by increased formation of ROS (Ognjanović *et al.*, 2003; Sinha *et al.*, 2008 and Kanter *et al.*, 2009). This study agreed well with the findings of (Stohs and Bagachi, 1995)

Catalase is a common enzyme found in nearly all living organisms exposed to oxygen. In the present study, catalase activity decreased in the liver extracts of the cadmium-treated group compared to normal rats which might be a result of metal deficiency. It is known that Cd decreases the levels of iron (Fe) in the liver (Jurczuk *et al.*, 2004) and because Fe is a component of the active site of catalase, a decrease in Fe might result in a decrease in catalase activity. And this agreed with the earlier studies (Al-Hashem *et al*., 2009).

GSH is a main non-enzymatic antioxidant in a living organism, which plays an essential role in coordinating the body's antioxidant defense process and is found in high concentration (Grosshan and Calvin, 1985). In this study, the level of glutathione in the liver tissue homogenate of the cadmium - treated group was reduced compared to control rats, which can be generated by cadmium due to their consumption in free radicals (Koyuturk *et al*., 2006 and Al-Hashem *et al*., 2009).

In the present work, we have to study the effect of Bee pollen and Selenium on liver damage induces by $CdCl_2$. It has been detected that selenium function through participates in the formation of selenoproteins, this element contributes in various biological processes such as antioxidant defense, immune responses and thyroid hormone production (Ognjanovic *et al*.,2008 and Tinggi,2008). Selenium tended to alleviate Got, Gpt plasma transaminases and ALP as demonstrated by us in the present study and this was in accordance with (El-Demerdash, 2004 and Soudani *et al*., 2011).The present results showed that treatment of animals Induced by cadmium chloride with selenium showed improvement in the level of direct and total Bilirubin. These results agree with (Hoffman and Heinz, 1998 and Fatma and Rezk, 2004)who reported that the selenium treatment caused a significant decrease in Direct and Total levels of Bilirubin

Which indicated that the Selenium has a protective effect on liver against Cd-induced toxicity by increasing antioxidant enzymes activities and decreasing MDA (Newairy et al., 2007).

The results of this study, Treatment with Se, were very effective in preventing oxidative damage caused by cadmium chloride, which led to a significant reduction in the level of MDA in the liver tissue. This can be explained by Se's important role in the prevention of lipid peroxidation and tissue integrity and function. However, treatment with selenium significant prevented the $CdCl_2$ induced depletion of GSH in liver indicating the antioxidant effect of selenium. Antioxidant enzymes and CAT is responsible for the catalytic decomposition of hydrogen peroxide to molecular oxygen and water (Aebi, 1984 and Abdulaziz *et al*., 2011). the activities of this antioxidant enzymes were a significant increase compared to the rats treated with cadmium chloride, and were very similar to the values noted in normal rats.

The present study showed that the treatment of rats Induced by CdCl2 with bee pollen showed

improvement in the level of serum GOT, GPT, alkaline phosphatase , protein , albumin and (direct and total) Bilirubin. It can be concluded that, groups of bee pollen for successive days treatment resulted in significant decrease in the level of GPT, GOT, ALP enzymes and a significant increase in Albumin and protein in serum of rats induced cadmium chloride when compared with the control animals. On the other hand, the activities of GOT, GPT, ALP *and (Direct_ Bilirubin)* are still higher than the normal animal, but the Albumin and *(Total_ Bilirubin)* improved and become near to normal animal.

Bee pollen is characterised by high antioxidative potential, which determines its biological activity (Kroyer *et al*., 2001 and Leja *et al*., 2007).

In the present study showed that the liver MDA concentration was significantly decreased on the other hand GSH and catalase were a significant increase when compared with control animals. But when compared with the normal rats the results indicated that MDA, GSH and catalase concentration were improved and become near to normal animal. The antioxidant effects of bee products have previously been confirmed by several reports (Moreira *et al.,* 2008, Ahn *et al.*, 2009) . Bee pollen has been reported to have relatively strong antioxidant effects, especially against hydrogen peroxide and superoxide radical (Nakajima *et al.,* 2009) and had the ability for inhibition of nitric oxide production (Maruyama *et al.,* 2010).

Conclusion and Recommendation:

The results indicated that Bee pollen and selenium showed a good improving role against cadmium-induced hepatotoxicity, this protection is mediated either by preventing cadmium - induced decline of the antioxidant liver defense system or by their direct free radical scavenging activity. Thus, bee pollen and selenium supplementation containing several antioxidants can be important for people exhibited to low doses of cadmium.

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ARABIC SUMMARY

"تأثير السيلينيوم وحبوب لقاح النحل ضد التسمم المناعى والكبدي الناجم عن الكادميوم في ذكور الجرذان البيضاء"

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الكادميوم هو عنصر سام ويؤثر على وظيفه العديد من أعضاء الجسم ، وقد صممت الدراسة الحالية لدراسة دور حبوب اللقاح والسيلينيوم ضد التسمم المناعى والتسمم الكبدي المستحث بواسطه كلوريد الكادميوم في ذكور الجرذان البيضاء

تم استخدام أربعين (40) من ذكور الجرذان البيضاء للتجربة. المجموعة الأولى تم حقنها بمحلول ملحي ، المجموعه الثانيه تم حقنها (1مجم / كجم من وزن الجسم) من كلوريد الكادميوم يوميا، المجموعه الثالثه تم حقنها (1مجم / كجم من وزن الجسم) من كلوريد الكادميوم ثم حقنت بجرعة مقدارها (0.5 مغ / كغ / يوم). من السيلينيوم. المجموعه الرابعه تم حقنها (1مجم / كجم من وزن الجسم) من كلوريد الكادميوم ثم تلقيت لقاح النحل فمويا بجرعة مقدارها (10 جم / كجم / يوم). تعرضت الحيوانات للعلاج مرة واحدة يوميا لمدة 10 أسابيع. بعد اليوم الأخير من العلاج ، تم ذبح الحيوانات وعينات الدم التي تم جمعها للتحاليل الدموية والمصل المفصولة للتحاليل البيوكيماوية الخاصة بالكبد المصلية وفصل الكبد لتحاليل الإجهاد التأكسدي. أظهرت النتائج التي تم الحصول عليها في هذه الدراسة زيادة معنوية في ($P> 0.05$) في WBCs و Monocyte و Lymphocyte و Granulocyte و IgG و IgM و Aspartate aminotransferase (AST) و alanine transferase (ALT) والفوسفاتيز القلوي (ALP) ، (الإجمالي- المباشر) البيليروبين و المالونديالدهيد (MAD) ، بينما أظهر انخفاض معنوي في ($p> 0.05$) في الألبومين والبروتين ، Glutathione (GSH) ، و superoxide dismutase (SOD) و Catalase (CAT)) في المجموعة المعالجة بـ CdCl2 فقط عند المقارنة مع المجموعة العادية. ومع ذلك ، فقد أظهرت تأثيرات لقاح النحل والسيلينيوم على السمية المناعية والإصابة الكبدية بسبب الإجهاد التأكسدي الناجم عن الكادميوم انخفاضاً ملحوظاً في ($p> 0.05$) في WBCs و Lymphocyte, Monocyte ، وأنشطة IgG فيما عدا Granulocyte و IgM لا تتأثر أيضاً كما أظهرت الأسبارتات ناقلة الأمين (AST) ، ألانين ترانسفيراز (ALT) ، الفوسفاتيز القلوي (ALP) ، البيليروبين (Total- Direct) و مالونديالديهيد (MAD) انخفاضاً ملحوظاً في ($p> 0.05$) ، في حين أن البروتين الكلي ، ألومين ، الجلوتاثيون (GSH) وأظهرت مادة ديسموتاز الفائق (SOD) ،والكاتليز(CAT) زيادة معنوية عند ($p> 0.05$) مقارنة بالمجموعة الضابطة. وخلص إلى أن لقاح النحل والسيلينيوم لديهم خصائص مضادة للأكسدة.